Departement für Nutztiere, Abteilung für Schweinemedizin
der Vetsuisse-Fakultät Universität Zürich

Direktor: Prof. Dr. Dr. h. c. U. Braun

A performance test for boar taint compounds in live boars

Inaugural Dissertation

zur Erlangung der Doktorwürde der

Vetsuisse-Fakultät Universität Zürich

vorgelegt von

Sveva Mattei

Tierärztin

von Onsernone-Crana, Tessin

genehmigt auf Antrag von

PD Dr. Xaver Sidler, Referent

Prof. Dr. P. Spring, Korreferent

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2. Abstract

In 2010, the Swiss service center for pig production SUISAG started a research project to develop a breeding program against boar taint. Together with the Swiss Commission for Technology and Innovation, some scientific partners and the retailer Coop, they set the goal of establishing a performance test for boar taint in sire lines and to develop a viable selection strategy against boar taint. This publication delineates the first part of the project. A biopsy device for collecting adipose tissue samples from live breeding candidates was developed. Due to the small core size (200 mg), special extraction methods for the analysis of androstenone, skatole and indole content were developed. Adipose tissue was sampled from the neck of 498 PREMO boars on 9 herd book farms (Swiss terminal sire line; age 128–287 days, weight 100 – 209 kg). Some boars were biopsied twice or three times, so that a total of 637 samples were available for analysis. Chemical analysis (HPLC) of adipose tissue samples revealed very low average concentrations of skatole (0.03 µg/g) and indole (0.03 µg/g). Androstenone concentrations were higher (0.66 µg/g), but still below the widely used critical value of 1.00 µg/g. 100 AI boars (Ø age 638.9 days, Ø weight 277.1 kg) had a mean androstenone of 5.01 µg/g. Behavioural reactions and bleeding were scored on five groups of boars (n=162). The correlation between 30 double fat samples of the neck region (H1, H2) was 0.93 for androstenone, 0.97 for skatole and 0.96 for indole.

3. Introduction

In many European countries surgical castration is performed on about 80–100% of male pigs with exception of Ireland and the United Kingdom, where castration is hardly ever practiced. In Portugal, Spain and Cyprus less than 40% of the male piglets are castrated. In the 27 EU countries, approximately 94 million boars per year are castrated surgically without anaesthesia (Fredriksen, 2009; Horgan, 2006).

The aim of castration is to avoid boar taint, which is an unpleasant odour, or flavour that can appear while cooking or eating pork meat. Boar taint is mainly caused by androstenone, skatole and indole. Androstenone is a lipophilic molecule produced by the leydig cells in the testes. Skatole and Indole are products of microbial metabolism of tryptophan produced in the caecum and colon (Andresen, 2006; Babol, 2004; Chen, 2007; Merks, 2009; Moe, 2009). Further originators of off flavors as oxidation of fatty acids (fishy/rancid off-flavor), sulphurous compounds produced from Maillard reactions (one of the main routes to flavour compounds during cooking, which occurs between amino acids and reducing sugars) and the presence of iron, are described (Campo, 2003; Mottram, 1998).

The castration of piglets without anaesthesia conflicts with animal welfare and ethics best practice, therefore researches of alternatives are recommended (FVE, 2009). An EU working group, consisting of representatives of European farmers, meat industry, retailers, scientists, veterinarians and animal welfare NGO's, committed to ban castration by January 2018. The signatories also declared to introduce analgesia and or anaesthesia by January 2012 as a first step (European Commission, 2010). In Germany prolonged analgesia with NSAID in quality systems is mandatory since 2009 (FVE, 2009). In 2007 Dutch parties within retail, industrial and food service sectors undertook efforts to ban castration by 2015 (PIGCAS-Congress, 2007). Supermarkets consigned to sell only meat from pork which has been castrated under anaesthesia after January, 2009. In Norway local anaesthesia for castration has been mandatory since 2002 and castration has to be performed by veterinarians. Castration without anaesthesia was scheduled to be banned in January, 2009; however, banning was postponed as acceptable alternatives were not yet available. The most frequent method used at the moment is local anaesthesia, as a

combination of intratesticular and subcutaneous injection of lidocaine with adrenaline (Fredriksen, 2006).

In Switzerland, anaesthesia and prolonged analgesia have been required for piglet castration since January 2010. Approximately 1.3 million male piglets per year are castrated under general anaesthesia either with isoflurane and additional preoperative prolonged pain management with an NSAID by the farmers themselves or with a combination of ketamine, azaperone and butorphanole or meloxicam by veterinarians. Anaesthesia with isoflurane requires the acquisition of an anaesthesia machine and special schooling (EDI, 2008). Vaccination against gonadotropin-releasing factor (GnRF) and finishing intact boars are two promising alternatives to surgical castration. Sperm sexing is not ready for implementation in the next future.

Considering animal welfare, finishing intact boars is the best alternative to conventional castration (Kupper, 2008). Furthermore intact boars have a better feed conversion and a higher leanness compared to barrows (Bonneau, 2006; Fredriksen, 2009; Moe, 2009; Zamaratskaia, 2008). However there are some disadvantages of finishing intact males. Intact males may be more aggressive (Preinerstorfer, 2010; Rydhmer, 2010) and there is the necessity for separate housing for boars, as the presence of sexual mature females increases the maximum level of male androstenone level in the pen (Giersing, 2000).

Based on analyses of Pauly et al. (2009) in Switzerland 5.5% of two groups of slaughtered boars (mean slaughter weight 72 and 82 kg) had boar taint at time of slaughtering (androstenone > 1 µg/g and/or skatole > 0.2 µg/g) while other researches revealed a percentage between 5% and 50% of tainted boar carcasses depending on the breed, according to the androstenone level (Frieden, 2011; review by Zamaratskaia, 2009).

The availability of a fast, objective and reliable method to control boar taint at the slaughter line (“electronic nose”) and a solution for the problem of processing and marketing a larger amount of tainted meat are premises for boar fattening. Currently both conditions are not given.

An economical study in Switzerland showed that boar fattening is the most efficient, if the fraction of tainted animals is lower than 5%, compared to vaccination against GnRH, inhalation and injection anaesthesia (Raaflaub, 2008).

Androstenone and skatole, the main components of boar taint, are heritable (Ducro-Steverink, 2006; Tholen, 2010; Frieden, 2011). Androstenone concentration varies between breed (Babol, 2004; Frieden, 2011; Merks, 2009; Merks, 2010; Moe, 2007; Squires, 2006). In literature the heritability (h^2) of androstenone is mentioned to be between 0.25 and 0.87 (Frieden, 2011; Squires, 2006; Tholen, 2010). In a model calculation, which assumes a heritability of androstenone of 0.50, at least 4 to 6 generations (8 to 12 years) are necessary to reduce the frequency of boars with >1 µg androstenone/g fat from 20% to 5% (Frieden, 2011). The same results were calculated with a heritability of 0.2 to 0.4 in a further study (Ducro-Steverink, 2006). Both studies ignore a possible influence on other production parameter as paternal fertility (sperm motility, volume, concentration) or maternal fertility (live born piglets, first insemination age, weaning-conception interval).

An association study involving 275 SNPs in 121 genes and compounds related to boar taint, concluded that polymorphisms in CYP2E1, CYP21, CYP2D6, CYP2C49, NGFIB and CTNND1 might be used to reduce levels of boar taint without affecting levels of testosterone, estrone sulphate, 17β-estradiol or length of the bulbo urethralis gland (Moe, 2009).

Merks et al (2010) are describing a positive correlation of daily weight gain with androstenone, and a negative correlation with skatole. They illustrate no significant difference between groups with high androstenone compounds and groups of dam line males with low compounds concerning the age of first mating, total number of born piglets, weaning to conception interval and gestation length.

The heritability of skatole is lower (0.23–0.56) than androstenone (Frieden, 2011; Tholen, 2010), since it is also influenced by management factors such as hygiene and feeding (Chen, 2007; Pauly, 2009; Preinerstorfer, 2010). A correlation between the presence of androstenone and skatole in the fat, probably due to reduced skatole metabolism by increased sex steroid activity is described (Babol, 1999). In an *in vitro*

study in isolated pig hepatocytes, androstenone was able to repress the expression of cytochrome P450IIE1 (CYP2E1), the enzyme principally responsible for skatole metabolism (Doran, 2004).

There is currently no standardized method for measuring the risk that breeding candidates will produce tainted offspring. One possibility is to measure boar taint compounds directly in the live breeding candidate. This allows early identification of low-risk boars, because performance information is immediately available and breeding organizations do not have to wait until data on large numbers of progeny have been collected. Furthermore, direct performance testing of the breeding individual allows discrimination between members of the same litter. Until now, this method has not been formally discussed.

The aim of this project was developing a standardized method for measuring the risk that breeding candidates will produce tainted offspring in the Swiss PREMO® terminal sire line by direct performance testing of the breeding individual analyzing small neck fat samples collected from male candidates via biopsy.

Successful phenotypical selection of low concentration of androstenone in the end-product would allow pig producers to move towards production of finishing boars.

In the second part of the project the correlation with breeding values of PREMO® boars with different levels of androstenone was evaluated. The third part assessed a breeding and selection strategy against boar taint with the Swiss PREMO® Large White (Baes, 2012).

This publication describes the development of a performance test for boar taint compounds in live boars using small fat samples collected via neck biopsy.

4. Material and methods

All methods implemented in this study were deemed in accordance with the Animal Welfare Act through the Swiss Federal Veterinary Office (Schweizerischer Bundesrat, 2008).

4.1 Development of a biopsy device

A captive bolt device, similar to the method used by Topigs in Beuningen, NL (Merks and Westerhof, personal communication, 2010), was developed for the extraction of about 100–200 mg of neck fat, using a steel rabbit stun gun (Kaninchenschlachthilfe „Finito“, Klaus-Gritsteinwerk GmbH & Co, Postfach 2180, D-32221 Bünde), modified by Jossi Orthopedics CH-8546 Islikon. The original compression spring was substituted with a stronger compression spring with FN 248 (Gutekunst + Co.KG Federnfabriken, Metzingen, Germany) and a fixed grip with a cylinder shape (Ø 34 mm x 21 mm), dismountable with a 4 mm hexagonal socket screw key was added (Figure 1). The reusable biopsy needle (developed by Jossi Orthopedics) measured 65 mm and had a diameter of 7 mm at the base and 6 mm at the apex. To allow the extraction of the samples from the side of the needle, a window (20 x 6 mm) was made 20 mm from the sharpened end, which is cut at an angle of 30 degrees. A metal wire of 0.4 mm in diameter was fixed with two holes inside the needle 7 mm from the sharpened end, to ensure fixation of the tissue sample in the needle (Figure 2).

Figure 1. The biopsy device for the extraction of tissue samples is equipped with a compression spring and a dismountable cylindrical grip.

Figure 2. The needle with a window for the extraction of tissue samples, a slightly tapered apex and a metal wire for the fixation of the tissue samples.

4.2 Biopsy location

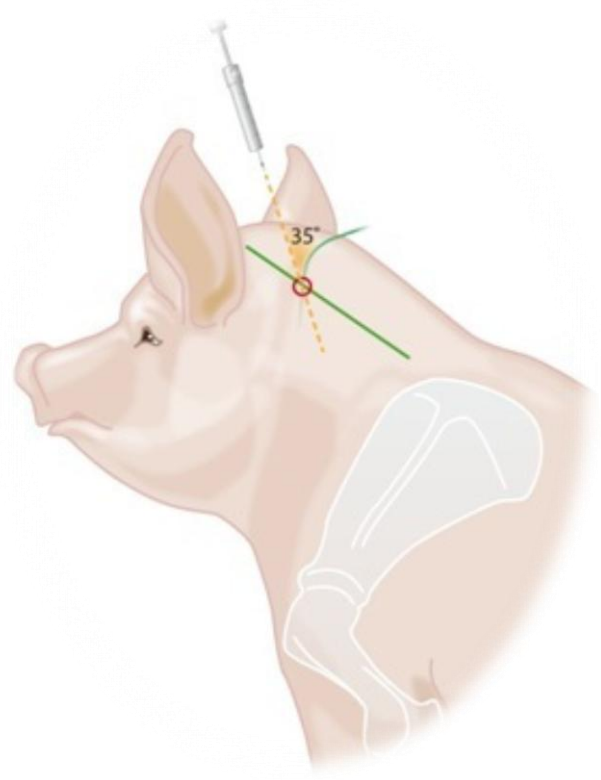

Figure 3. Biopsy location at the neck of the boar

We restricted the biopsies to the neck region in the middle of an imaginary line between the upper ear border and the shoulder. Multiple insertion angles (30°, 45° 60° and 90°) were tested on dead animals. The best angle in order to gather the largest amount of fatty tissue appeared to be about 30–35° to the skin (Figure 3), thus in the further steps only this angle was used. The biopsies were conducted by the project veterinarian and were taken without general or local anaesthesia and without fixing the boars with a nose sling.

The subsequent trials were divided in five steps: in *step 1* we took fat samples from the neck (H1, H2) and loin (L) region (weight ~ 200 and 300 mg) at the slaughterhouse on carcasses of Swiss Large White dam line boars. These samples were sent in addition to the normal loin samples (Lsuisag) to the laboratory of the Research Station Agroscope Liebefeld-Posieux ALP in order to develop an analysis

method for androstenone, skatole and indole with very small amount of fat, and to improve the biopsy device and test it on carcasses.

4.3 Scoring of the reaction of the boars and wound healing

In *step 2* four live boars (*group 1*) were sampled immediately before slaughter to score their reactions and to assess blood loss according to the scores in Table 1. The boars were sampled in an animal cage weighing scale without anaesthesia, so that the weight could be recorded simultaneously.

	Vocal (0–3)	Motoric reaction (0–3)	Blood loss (0–3)
0	No vocal reaction	No movement	No blood loss
1	Slight vocal response/ short scream	Small movement/twitch	Maximally 1 ml blood loss
2	Moderate vocal reaction/ scream	A few steps, a little jump to the side	Approximately 1–5 ml blood loss
3	Severe and permanent screaming	Intense movements/ flight attempt	Severe blood loss (> 5 ml)

Table 1. Scoring table of the reactions and blood loss of the boars because of the biopsy

In *step 3,* 14 dam line boars were sampled 10 days before slaughter, to observe wound healing in addition to the reactions. *Group 2* consisted of 8 boars in single pens. Their biopsy location was disinfected with 80% ketonyzed ethanol before taking the biopsy, and treated afterwards with an antibiotic spray (Chlor-Tetracyclin-Spray Stricker ad us. vet. http://www.stricker-ag.ch). In *Group 3* (6 boars in group pen) the biopsy location was cleaned with alcohol before taking the biopsy, but no further treatment was done. The animals of group 2 and 3 were free to walk and weighted separately. For the monitoring of wound healing the biopsy location was observed daily according to predefined scoring guidelines (Table 2).

Scale	0	1	2	3
Discharge	None	drops	with blood	with pus
Swelling	None	a little	moderate	severe
Skin redness	None	light	red	dark red

Table 2. Scoring table of skin reaction after the biopsy

In *step 4* two pilot PREMO® breeding farms (*group 4*, n=44 boars with > 90 kg live weight (LW)) and 100 PREMO® boars of the Suisag stud station located in Knutwil (*group 5)*, were sampled for the test trial in order to score behavioural reactions and wound healing in practical environment.

In *step 5* the PREMO® boar-candidates of 9 breeding farms (> 100 kg LW) were biopsied in connection with the field test for breeding values (*group 6*, n=498), by four technicians who had a special formation by the performing veterinarian, which included a theoretical and a practical part (20 samples on cadavers and 20 live animals under supervision). The boars, if still present at the farm, were sampled repeatedly (maximally three times).

4.4 Cleaning and transport of the biopsy device

Before taking a biopsy the needle was disinfected in 80% alcohol. After visiting a farm or a slaughterhouse the whole apparatus was washed and disinfected with alcohol, occasionally sterilized with gas. The fat samples were transported via night express to the ALP laboratory in Posieux in 1.5 ml Crio Tubes (www.tpp.ch) or 1.8 ml Nunc Crio Tubes with External Thread (www.nuncbrand.com) in a refrigerated styrofoam box with cooling pads.

4.5 Analysis of Androstenone, Skatole and Indole

Boar taint compounds were determined at the laboratory of the Research Station Agroscope Liebefeld-Posieux ALP by HPLC via fluorescence detection (Ampuero Kragten, 2011). The method was developed for very small amounts of liquid fat. Adipose tissue was separated mechanically from the rest (skin, muscular tissue,

blood and hairs) during the thawing step. Liquid fat (LF) was obtained from adipose tissue during a heating process in a microwave oven. Furthermore, due to the high variability of the utile fraction of biopsy samples, the analytical method included 2 variants: a) for samples > 70 to 100 mg LF and b) for samples ≤ 70 mg LF. Whenever possible, a double determination was allowed with at least one of the replicates with variant a).

4.6 Statistics

For the statistical evaluation of correlations between the samples we used Microsoft Office Excel 2007. Boar taint data were log transformed to achieve a normal distribution.

5. Results

5.1 Boar reactions and blood loss

In order to measure the reactions to the shot biopsy, we scored 18 boars of the performance station in Sempach (*groups 1–3*), 44 boars of the two pilot breeding farms (*group 4*), and 100 boars of the boar stud (AI boars, *group 5*).

Score/Group no.		1 (n=4)	2 (n=8)	3 (n=6)	4 (n=44)	5 (n=100)
% Vocalisation	0	50	50	66.67	90.91	95
	1	25	37.5	33.33	9.09	3
	2	25	12.5	0	0	1
	3	0	0	0	0	1
% Motoric reaction	0	25	62.5	66.67	93.18	98
	1	50	37.5	33.33	6.82	2
	2	25	0	0	0	0
	3	0	0	0	0	0
% Blood loss	0	50	75	50.01	79.55	84
	1	25	25	33.33	18.18	13
	2	25	0	16.66	2.27	2
	3	0	0	0	0	1

Table 3. Percentages of the reactions in different environments of group 1 – 5.

In Table 3, the scores of different reactions are shown. A total of 70% of all scored boars had no reaction to the biopsy (86% had no vocal and motoric reaction). The reactions of the first three boar groups (n=18, av. weight 110.2 kg) were rather mild, even if in some distress due to the unfamiliar condition as walking towards the slaughter house (*group 1*) or the presence of an unfamiliar person. The vocal reactions were slight in 6 of 18 boars and moderate in 2 boars; the others didn't show any vocal reaction. The motoric reaction consisted of a small twitch by 7 boars and a jump to the side by 1 boar; the remaining boars didn't move. Blood loss was negligible: 6 boars lost < 1 ml blood, one boar lost < 5 ml of blood.

4 of the 44 boars scored (mean weight 107.6 kg), showed a vocal reaction grade 1 (9%), 3 showed a motoric reaction score 1 (7%) and 1 boars had bleedings of grade 1 (18%), 1 had a bleeding of grade 2 (2%).

In g*roup 5* only a total of 24 % had a score > 0.

5.2 Observations of the wound healing

Results of wound healing are shown in Table 4. The biopsy localization of the animals of group 2 and 3 was controlled daily from day one to day 10. Boars of *group 2* (antibiotic spray, housed in single pens) had a quicker wound healing process compared to *group 3* housed in group pen without antibiotic spray particularly comparing the duration of skin redness and swelling.

The wound discharge was clear and serous at almost all occasions (score 1), sometimes mixed with a small blood residual (score 2).

For the *groups 4 and 5* we didn't get any negative reports regarding wound healing from the owners.

Day/healing process	Group 2 (n=8)	Group 3 (n=6)
Day 1		
discharge	1 x score 2, 3 x score 1	2 x score 1
skin redness	0	0
swelling	6 x score 1	3 x score 1
Day 2		
discharge	1 x score 1	0
skin redness	0	3 x score 1
swelling	1 x score 1	6 x score 1
Day 3		
discharge	0	0
skin redness	0	5 x score 1
swelling	0	5 x score 1
Day 4		
discharge	0	0
skin redness	0	0
swelling	0	1 x score 2, 3 x score 2
Day 5		
discharge	0	0
skin redness	0	0
swelling	0	2 x score 1
Day 6 - 10		
discharge	0	0
skin redness	0	0
swelling	0	0

Table 4. Wound healing process. Group 2 (n=8) in single cages with disinfection (alcohol) before sampling and tetracycline spray after sampling, group 3 (n=6) in group pens and without local treatment after sampling.

5.3 Correlation liquid fat amount – biopsy angle

The results of the sample weight and the amount of liquid fat (LF) according to the biopsy angle are visible in Table 5. The sample size and amount of LF was decreasing with growing angle. These results led us to the decision to use an angle of about 30–35° for our standard biopsies.

	Dead 30°		Alive 45°		Dead 90°		Dead 60°	
Animal	Sample[1]	LF[2]	Sample[1]	LF[2]	Sample[1]	LF[2]	Sample[1]	LF[2]
1	635.1	157.8	358.1	99.6	485.2	162.6	437.6	118.9
2	680.5	237.2	595.6	189.4	609.7	180.0	642.1	188.3
3	448.9	106.3	394.3	73.7	394.8	75.7	390.2	90.2
4	408.7	126.5	326.8	88.8	423.6	129.2	382.5	108.2
Mean	543.3	157.0	418.7	112.9	478.3	136.9	463.1	126.4
St. Dev.	134.5	44.6	121.1	45.1	95.4	39.8	121.8	37.2

Table 5. Total mass (mg) of the first four biopsy samples[1] (skin, muscle tissue, adipose tissue) and the correspondent liquid fat[2] (LF) amount harvested from 4 animals at different angles (e.g. 30°, 45°, 60°, 90°). The samples at 45° were taken from live animals.

5.4 Correlation between fat samples

5.4.1 Correlation between two adjacent samples of the neck

Thirty carcasses of Swiss Large White dam line boars were sampled twice at the neck: the correlation coefficient (R^2) between the results of two adjacent neck biopsies (H1 and H2) taken simultaneously is 0.93 for androstenone, 0.97 for skatole and 0.96 for indole (Figure 4).

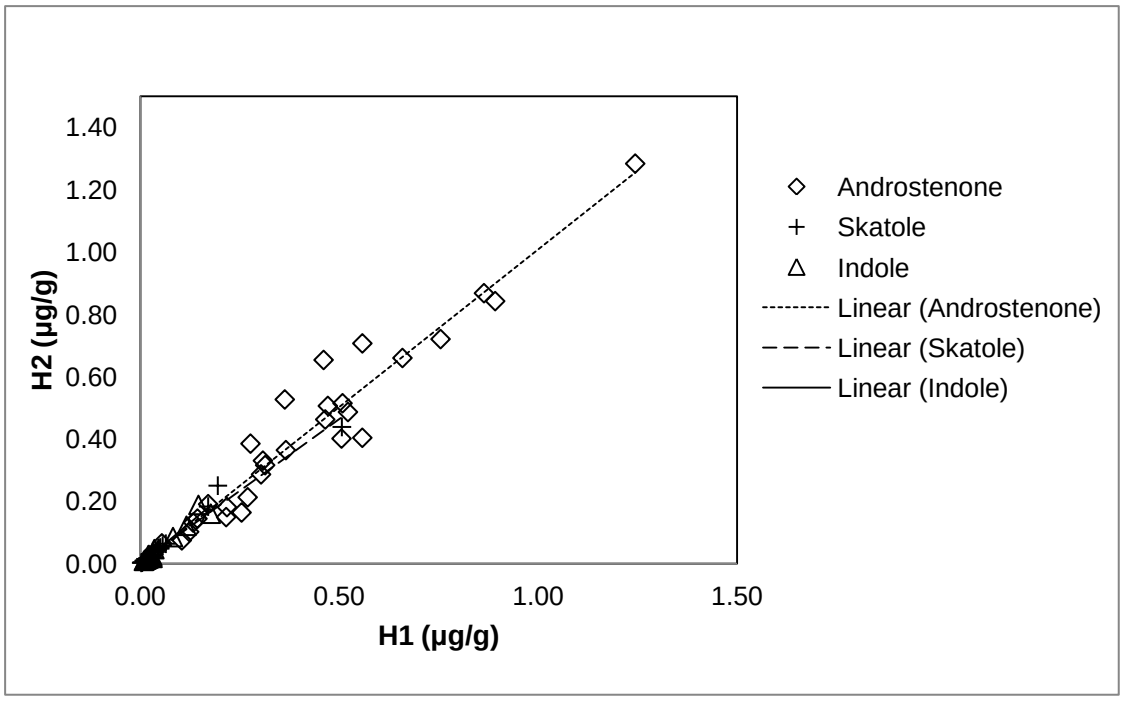

Figure 4. Correlations between the values of androstenone, skatole and indole in the two neck samples taken manually from Swiss Large White dam line boars at the slaughterhouse (H1 and H2) (n = 30).

5.4.2 Correlation between samples of loin and neck

On 14 carcasses we tested the correlation of androstenone from three hand token small fat samples (H1, H2 neck samples, L loin sample) with the results of larger amounts of loin fat used for standard androstenone measuring (Lsuisag), in order to allow the ALP laboratory to calculate a conversion factor. As represented in Figure 5 the correlation (R) between the three small samples with the larger Lsuisag sample was lower for the androstenone values of the neck biopsies (both 0.94) compared to the loin biopsy (0.98). For skatole and indole the correlations were comparable (mean 0.99).

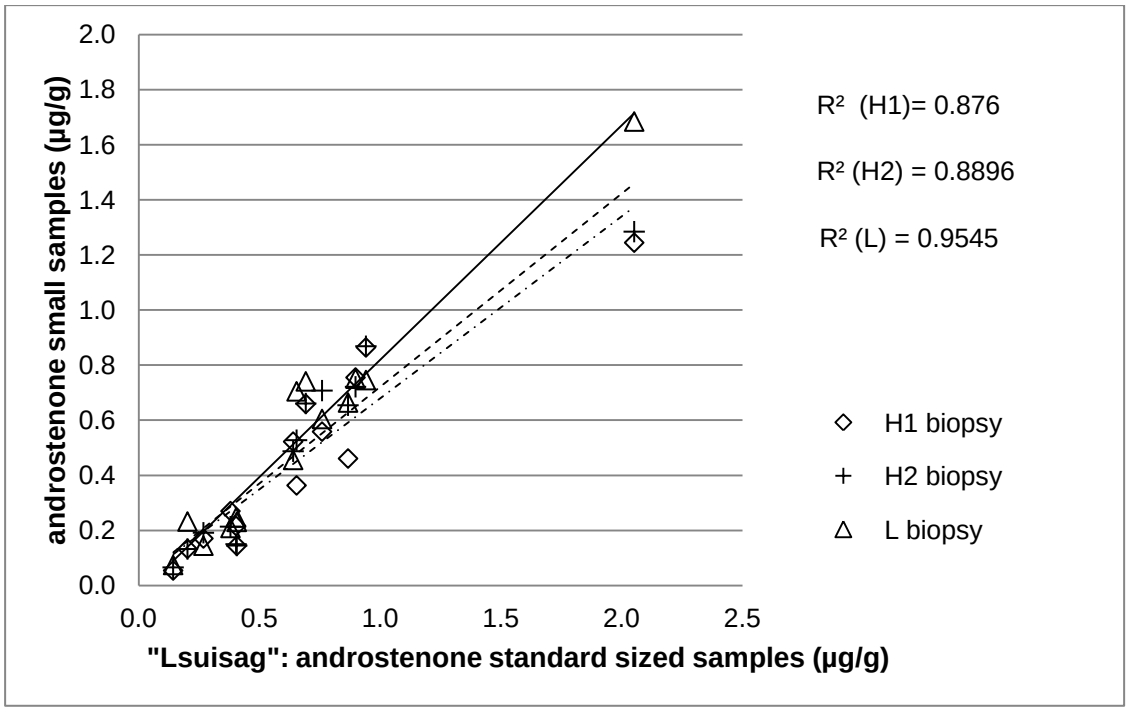

Figure 5. Correlations of the androstenone values of the loin fat ("Lsuisag"), and the values of three small samples (200–300 mg): H1 and H2 are neck biopsies, L is the loin biopsy (n=14).

5.5 Androstenone, skatole and indole values of 100 stud boars

The androstenone values of 100 breeding boars in a boar stud were much higher compared to the breeding candidates and showed a larger scatter. The mean from 100 boars was 5.01 µg/g androstenone. On the other hand for skatole and indole, results were comparable with the results of the breeding candidates.

n = 100	Mean	s.d.	Min.	Max.
Androstenone (µg/g)	5.01	3.83	0.00	14.25
Skatole (µg/g)	0.02	0.03	0.00	0.23
Indole (µg/g)	0.08	0.11	0.01	0.75
Age (days)	638.9	347.9	216	1807
Weight (kg)	277.1	70.1	145	447

Table 6. Mean, standard deviation, minimum and maximum of 100 boars of the boar stud sampled all the same day.

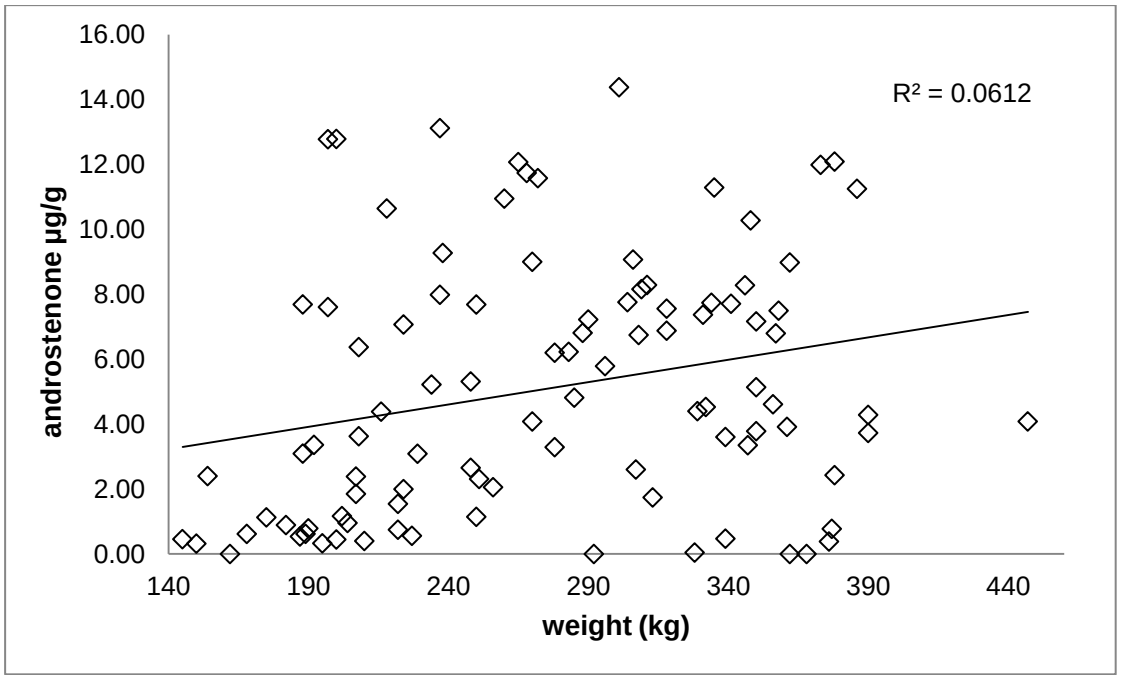

Figure 6. Correlation between results of the androstenone values and body weight of n=100 boars of the boar stud (group 5).

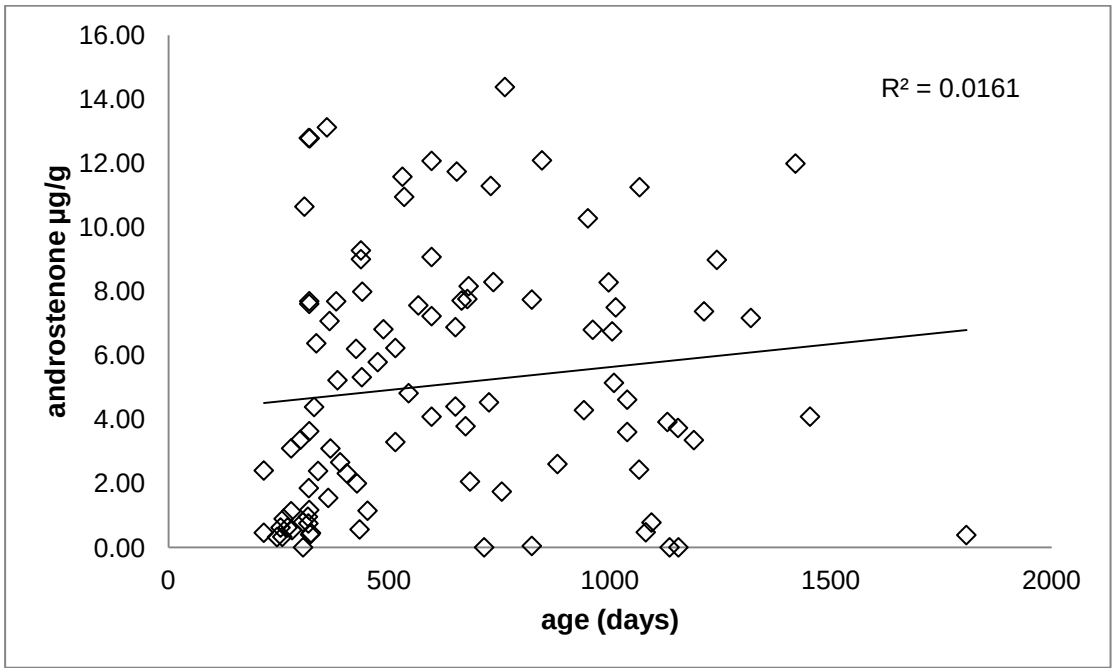

Figure 7. Correlation of androstenone values to the age of 100 boars of the boar stud.

5.6 Results of the PREMO® Boars during the field test for breeding values

5.6.1 Results of all boars

The means, standard deviations, minima and maxima of the results of 637 fat samples of 498 Swiss PREMO® breeding candidates are displayed in Table 7:

n = 637	mean	s.d.	min.	max.
Androstenone (µg/g)	0.66	0.64	0.00	5.59
Skatole (µg/g)	0.03	0.05	0.00	0.63
Indole (µg/g)	0.03	0.04	0.01	0.40
Age (days)	171.6	22.5	128	287
Weight (kg)	117.3	14.9	100	209

Table 7. Mean, standard deviation, minimum and maximum of the results of the 637 PREMO® boars sampled on 9 farms.

Concerning androstenone, 8 boars (1.26%) had a value >3 µg/g, 114 boars (17.9%) had a value between 1 and 3 µg/g, 171 (26.84%) had a value between 0.5 and 1 µg/g, and 344 (54%) had a value < 0.5 µg/g. Concerning skatole 98.43% of the boars had a value lower than 0.2 µg/g, while 98.59% of the boars had an indole value < 0.2 µg/g.

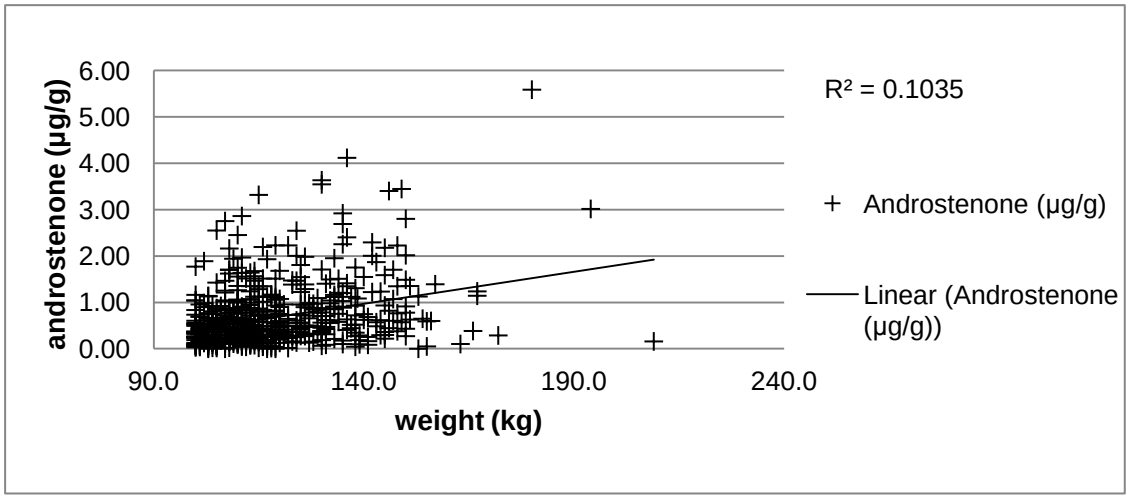

Figure 8. Androstenone (µg/g) in relation to the weight of 637 samples of PREMO® boars.

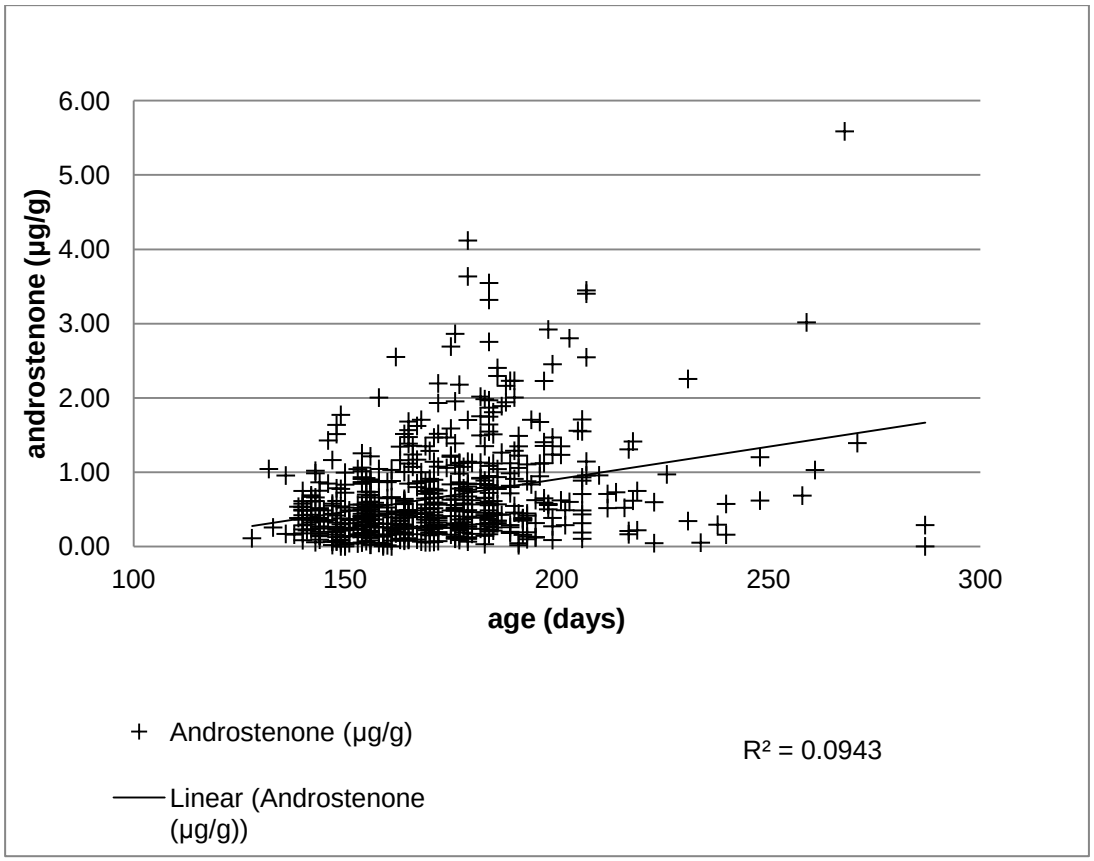

Figure 9. Androstenone (µg/g) in relation to the age (days) of 637 PREMO® boars.

The correlation coefficient of skatole and indole of the 637 fat samples of 498 Swiss PREMO® breeding candidates was $R^2 = 0.6407$ (Figure 10).

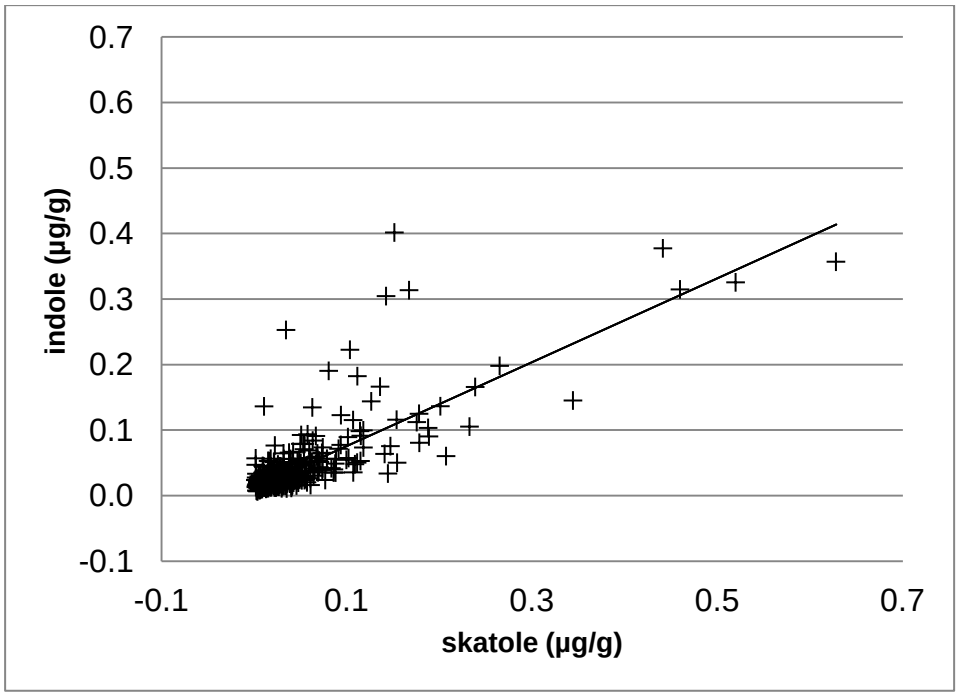

Figure 10. Values of skatole (µg/g) and indole for 637 PREMO® boars.

5.6.2 Values of the boars with a body weight <120 kg

425 boars had a body weight < 120 kg: of these samples 1 boar (0.24%) had an androstenone value >3 µg/g, 58 boars (13.65%) had a value between 1 and 3 µg/g, 110 (25.88%) had values between 0.5 and 1 µg/g, 256 (60.24%) had an androstenone value < 0.5 µg/g. Concerning skatole 98.59% of the boars had a value < 0.2 µg/g, for indole 99.06 % of the samples were under 0.2 µg/g.

5.6.3 Correlation between repeated samples of the same animal

The results of 128 boars, sampled twice (over a period of 13–70 days) are visible in Figure 11. The correlation coefficients (R^2) are 0.41 for androstenone, 0.62 for skatole and 0.51 for indole. 75 of these boars had a time interval between the two samples of ≤ 15 days. In this case the correlation coefficients are major: 0.47 for androstenone, 0.82 for skatole, and 0.62 for indole.

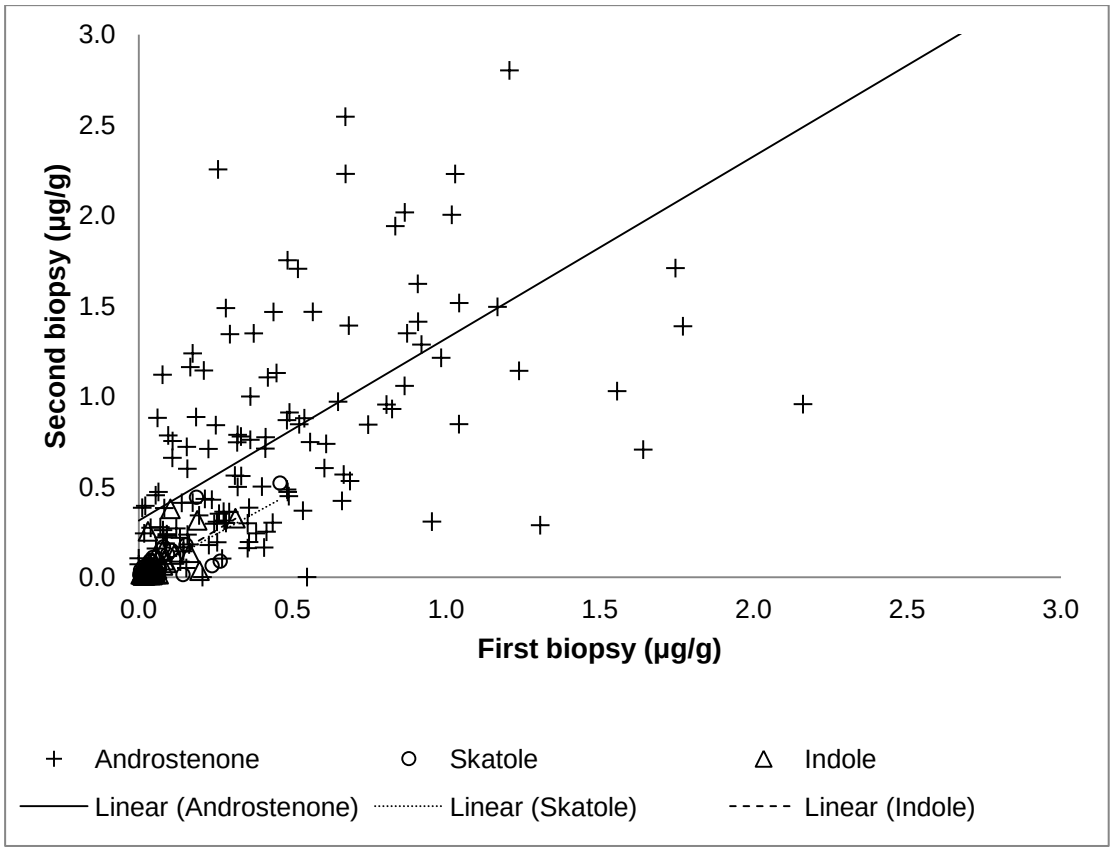

Figure 11. Results of 128 boars sampled twice over a time of 13–70 days.

6. Discussion

Animal protection and sustainability. Castration of piglets without anaesthesia or pain management conflicts with animal welfare and ethics best practice. Even anaesthesia with isoflurane is stressful and therefore researches of alternatives are recommended (FVE, 2009). Consumers, if informed, choose animal friendlier products (Hofer, 2008). Genetically reducing boar taint using low-taint breeding lines is considered the most sustainable and economic long-term alternative to surgical castration of male pigs. Owing to the high heritability of the main boar taint components (androstenone, skatole and indole), breeding is an excellent tool for reducing the number of tainted carcasses. To incorporate boar taint into breeding programs, standardized performance testing is required.

Biopsy. The objective of this study was to develop an easy performance test for the main boar taint compounds on live breeding candidates. The use of a biopsy device for measurement of boar taint compounds was introduced by Lundström et al. in 1973 (Lundström, 1973). Since then, a number of research groups have used biopsy methods to measure boar taint compounds for experimental purposes (Bonneau, 1987; Keller, 1997; Sellier, 2000). In literature biopsy sampling was described on the long back muscles without pain elimination and simple wound treatment (Geverink, 1999; Scholz, 2002). In the study of Geverink et al., 1999, 70–90 % of the pigs vocalized when the biopsy was taken, and all pigs flinched in response to the biopsy. Therefore, we used a modified biopsy device to extract small tissue samples (300–600 mg) from breeding candidates to earn 100–220 mg liquid fat. In contrast to Geverink et al (1999) we biopsied the upper neck instead of the loin region. The insertion angle of about 35° enabled minimal muscle lesion. Important anatomical structures such as major blood vessels and nerves could be avoided. Injury of muscle tissue via biopsy probably causes an increased pain reaction and blood loss, as the number of blood vessels and nerve endings in muscle tissue is higher than in adipose tissue. In addition, in the case that the animal had to be fixed by a nose-sling, this location can be controlled very well. The chosen method to test live animal is minimally invasive, fast and easily practicable on a farm.

Reactions. The reactions of the boars could be caused from pain or fear. We didn't investigate further to distinguish between these alternatives. 86% of the tested animals didn't show any motoric and vocal response when biopsied. Stud boars *(group 5)* showed a minor percentage of response, because major age and the increasing thickness of fat layer which minimizes contact with muscular tissue. Psychological components can also play a role, because pigs as intelligent mammals are influenced by experience (Gabor, 1999; Laughlin, 2000). Our biopsy device had a slightly thinner needle than that used in the study by Geverink et al. (1999), which minimized the invasiveness of sampling. Possibly a further pain influencing factor is the higher strength of the compression spring. Members of Swiss animal protection and the cantonal veterinary services evaluated a biopsy personally and confirmed that neck biopsies may be performed routinely without requiring local or general anaesthesia by a technician with a proper instruction.

Wound healing. The wound healing progress was unproblematic. The longer lasting duration of swelling and redness of *group 3* (animals kept in a group pen without the use of an antibiotic spray) compared to *group 2* (single pens with antibiotic treatment) is a sign of a slightly increased inflammatory reaction. The biopsy location was selected to be in a place assumed to be less likely contaminated with faeces. Possible explanations of the increased inflammation in *group 3* could be, in addition to the effect of the antibiotic spray, the minor amount of space per animal and its consequences. The decision not to use antibiotic spray to treat the biopsy location after taking the biopsy, was taken as the use of antibiotics selects resistant bacteria (van de Sande-Bruinsma, 2008; Teuber, 2001).

Correlations. In the first step of the project, multiple samples were collected from the neck. Figure 4 shows that the correlation coefficients are very high (R^2 = 0.93–0.97), a sign for a high repeatability of the testing methods even if the samples are very small. Ampuero, in an interlaboratory comparison of methods to measure androstenone in pork fat, showed that the method of sample preparation had an unequivocal effect on the correlation of the results: with the same lyophilised samples pairwise correlations between laboratories ranged from 0.92 to 0.97 (Ampuero Kragten, 2011). Whenever possible, two replicates from each biopsy core were analysed. The lower correlation of the androstenone values between samples of the

neck with the back fat biopsy suggests a different distribution of androstenone in the various parts of the body.

Androstenone, skatole and indole levels. Androstenone levels exceeding 1 µg/g were found in 13.88% of the boars (weight 100–120 kg). These results are lower compared to the results of other researches (resume in Bonneau, 2006). Skatole levels were very low, as 98.59% of the animals between 100 and 120 kg of weight had a value below 0.2 µg/g. Skatole levels can be reduced by diet, for example by adding potato starch (Chen, 2007; Pauly, 2008), and should not be the major issue when producing intact boars. The androstenone value of the 100 tested boars of the boar stud increased slightly according to age and body weight (R^2_{age} = 0.01, R^2_{weight} = 0.06), while the correlation was higher in the younger boars (R^2_{age} = 0.09, R^2_{weight} = 0.10). Despite of advanced age and weight some boars showed very low androstenone values and were still used for breeding purposes.

Many studies report a surge in boar taint at puberty (e.g. Willeke, 1987; Sellier, 1988). Zamaratskaia and Squires (2009) distinguished between three types of boars: early maturing boars with high androstenone, late maturing boars with high androstenone and boars with low androstenone. Therefore, performance testing should be conducted only after late maturing boars differentiate from low taint boars with regard to boar taint, which ensures that the trait being selected for is boar taint and not late maturity. Because slaughter weights in Europe are relatively low (100 to 130 kg live weight), boar taint components in late maturing males may not have reached their peak at this weight (in our study, 14.1% of all observations exceeded the androstenone threshold of 1 µg/g fat at live weights between 100 and 130 kg). Therefore, a compromise is needed in which performance testing is conducted late enough to ensure that later maturing boars differentiate from low-taint boars but early enough to represent the target slaughter weight. A further advantage of early performance testing is that tested boars not selected for breeding can still be slaughtered without deductions for high weight.

Heritability. The difference in the distribution of skatole and indole in different breeds suggests that also these substances are heritable (Babol, 2004). This could be

induced by a relationship between the metabolism of skatole and steroids (Andresen, 2006; Babol, 1999) and by the values of skatole and indole being induced by androstenone, or by a heritability linked to separate genes. In the analysis conducted by C. Baes in this project heritability of skatole and indole were higher than that of androstenone

7. Conclusions

Accurate and precise sampling and analysis methods for quantifying androstenone, skatole and indole in small adipose tissue samples obtained from boars over 100 kg live weight were developed. Biopsy wounds healed quickly, even without post biopsy antibiotics, and behavioural observations of biopsied animals showed very little pain related response to the biopsy.

The described biopsy device is useful in future to gain insight into the pubertal development of boars. Investigating the trend of individual boar taint components over time in live animals could help in understanding the relationships between puberty, age, weight and boar taint compounds. Future research should include further investigation of correlated effects on economically important traits, especially in dam lines.

This study is a step towards a more ethical and animal friendlier pork production. Though, there is still a long way to go before we reach animal friendly production systems, as large scale meat production in a limited space, affiliated with an increasing product demand, make a great challenge to solve.

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